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Physiology of the Pollen of Zea Mays with Special Regard to Vitality

BY

DEMETRIUS ION ANDRONESCU

JUN 1915

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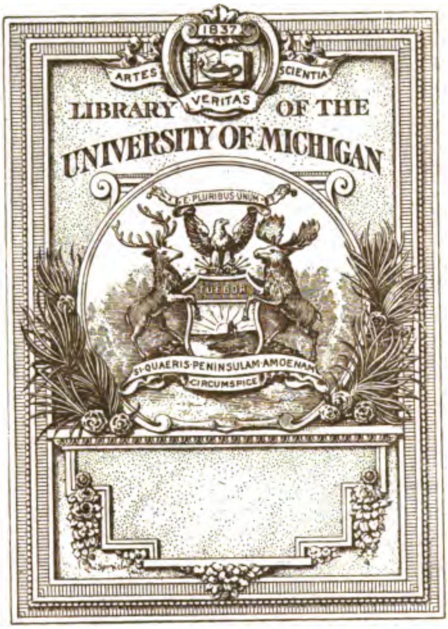
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THE PHYSIOLOGY OF THE POLLEN OF ZEA MAYS WITH SPECIAL REGARD TO VITALITY

BY

DEMETRIUS ION ANDRONESCU

Diploma of Capacity in Agronomy, Roumanian College of Agriculture, 1906.
Master of Science, University of Illinois, 1914.

U of M

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IN

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I. THE PROBLEM

The purpose of the following investigation is to ascertain the length of time that Maize pollen retains its viability and is capable of effecting pollination and fecundation.

In hybridization work it often occurs that the silks of one plant are not receptive just at the time the pollen is developed in the plant to be crossed with it. The question then is to determine how long the pollen will keep its vitality.

The work involves two questions: first, the necessary conditions for the germination of the pollen; and second, the storage of the pollen.

II. HISTORICAL

Van Tieghem (26), Hansgirg (9) and Lidforss (13) found that pollen of many species can germinate in water or moist air. Rittinghaus (22) and many others found that pollen of very many species would germinate in sugar solution with a concentration of between 1 and 40 per cent. Mangin (16), established that agar or gelatin added to sugar solutions can increase the percentage of germination. Max Pfundt (20) found that corn pollen can germinate in a solution of cane sugar in concentration of from 15 per cent. to 35 per cent., while other grasses require 20 per cent to 50 per cent. Kny, quoted by Martin (17), says that Lilium bulbiferum, Aesculus Hippocastanum, Robinia Pseud-acacia, Pisum sativum, etc., germinate better in cane sugar solution with gelatin. Jost (12), germinating pollen on parchment soaked in distilled water and dried on filter paper, concluded that the germination of the pollen of some grasses depends entirely upon the water supply. Hans Molisch (18), found that calcium malate, malic acid, citric acid, tartaric acid, saltpeter and asparagin added to sugar solutions had a slight stimulative effect upon the germination of pollen of many species. Lidforss (13), found that a small percentage of citric acid added to sugar solution induced the germination of pollen of some species of Erica and Menziesia, while a small percentage of calcium or potassium salts or a lack of aeration prevent germination in many species. Tischler (25) obtained good germination by adding diastase to sugar solutions for Solanum rostratum and some of the Melastomaceae, Liliaceae, Lythraceae and the genus cassia of the Leguminosae. Richter (21) and others found that many kinds of pollen which failed to germinate in sugar solutions readily sent out their pollen tube if fragments of stigma from the same or nearly related species were placed in the culture. Burck (2), observed that pollen of certain species of Mussaedena would germinate in distilled water if portions of the stigmas or if levulose were added. From the work of Molisch (18), Lidforss (13) and others, it is understood that Chemotropism of carbohydrates or proteins and negative aerotropism can influence the direction of pollen tubes. Duggar (5), found that "Pollen of Corn and some other grasses, also many sedges and rushes, germinates best in a moist atmosphere and these may be sown on a dry cover-glass inverted over a cell containing water." Martin (17), found that sugar solution containing agar or gelatin allows less bursting, and 2 grams to 5 grams of gelatin added to a 0.731 volume normal solution of sucrose gave the best medium for the pollen of Trifolium hybridum and Trifolium repens. McCluer (15), reported that corn pollen seemed to retain its vitality for several days if kept dry. Webber (27), observed that corn pollen retained its vitality at least two weeks. Jost (12) found that corn pollen is viable only two days under optimum conditions. Pfundt (20), states that pollen of corn remained viable only one day, alike in 30 per cent, 60 per cent, and 90 per cent moisture. Sandsten (23) found that tomato pollen required a slightly acid medium and that apple pollen retained its viability for six months. Booth (1), germinated grape pollen in New York three weeks after it had been gathered in California. Gernert (6), using fresh pollen on old silks, old pollen on fresh silks and the intermediate combinations, declared that "no reliable cases were found, including hybrids in all the groups and many varieties, in which shoots produced kernels when pollinated with pollen that has been stored 30 hours." Burt-Davy (3), says that "in the dry climate of South Africa, the corn pollen keeps well its vitality for three days, but after five days most of it is no longer viable." Experiments mentioned in the Experiment Station Record (24), states that pollen of roses may retain its vitality for 22 days; Clivias, at least three months, some hybrids for over a year; Cannas for fifteen days or more and Aucuba for ten days. Hartley (11), says: "The pollen from the tassel of a sucker possesses all the value possessed by the pollen of the stalk that produced the sucker."

III. THE TASSEL

The maize plant is monoecious, it bears the reproductive organs in separate flowers on the same plant, but the separation is not always complete for bisexual tassels are frequently found and stamens sometimes occur in pistillate flowers.

The appearance of the pistillate flowers on the central axis of the tassel, which often occurs in many varieties of corn, suggested to Montgomery (19), " that corn and teosinate may have had a common origin.

The tassel is arranged in the form of a panicle, the branches of which are shorter nearer the base. The size and shape of tassels are various in the same variety of corn. According to Gernert (6), the corn grown in the Illinois Experiment Station has many different forms of tassel which he was able to classify into twelve distinct artificial type groups.

The tassel consists of numerous branches bearing more or less distichous rows of staminate spikelets which are arranged in pairs, one pedicellate, the other sessile. Each spikelet is protected by glumes which enclose two florets. There are three stamens in each flower, mounted on short filaments but which, as the pollen matures, lengthen and push the pollen out of the sacs. Each anther consists of two sacs, attached side by side, and having an opening or pore at the lower end for the emission of the pollen. The glumes and anthers are variously colored green, reddish-green, dark red, etc., and often the glumes are striped longitudinally with magenta or pink.

According to Harshberger (10), in an ordinary corn plant, 2,500 pollen grains are formed in a single anther and eighteen millions are given as the number produced by each plant or approximately 6,000 to 8,000 pollen grains for every ovule to be fertilized.

Usually the maize is proterandrous, the pollen being produced before the stigmas are receptive, but sometimes it has been found that the maize may be proterogynous, or synacmous.

Collins (4), describes a variety of red pop-corn from Spain which was almost entirely proterogynous. Burt-Davy (3), suggests that "probable proterogyny is a breed characteristic. It appears to be constant in Arcadia Sugar-Maize and in Wills gehu (yellow flint) while proterandry is the rule in many dent breeds."

In the Roumanian varieties of maize called Pignoletto, Hangan and Common (all flint) we often find proterogny, the silks appearing one to three days before the pollen.

The flowering period in the field can be extended over ten days.

IV. DESCRIPTION OF THE POLLEN

The first staminate flowers to mature are those in the upper part of the axis or central spike of the tassel, the sequence being in both directions but mostly downward and in the branches, inward. Generally the anthers shed pollen only on the first day in which they appear.

In our personal work we observed that when the stamens are mature, the pollen is discharged through the pore at the lower end of the sacs with considerable force. The time of the blooming begins in the morning. For many days the writer made observations in the early morning and found a large number of tassels blooming and shedding the pollen by six o'clock, but by eight o'clock, so universal and profuse was the bloom that, seen through the reflection of the sun's rays, the very air appeared to be yellow with the clouds of flying pollen. Before noon there was a cessation of pollen spreading, until late in the afternoon when a few tassels were observed to bloom again.

The time required for complete blooming of the tassels is variable, some discharging all of their pollen in three days while others have been observed to cover a period of from four to eight days in the process.

In general when the stamens are matured, the pollen is discharged from the two sacs simultaneously, but the writer has noticed a number of variations from this rule, as, for instance, one sac would be quite emptied while the other had discharged but one-third or one-half its pollen. Sometimes the balance of the pollen would be emitted in the following hour, or days, or it would not be discharged at all. In some cases it was observed that the stagnation of the pollen inside of the sac was due to an improper dehisence of the pore of the sac while very many cases were unexplained.

Each grain of pollen is a separate cell consisting of a cell-wall of usually two membranes known as intine and extine, surrounding a mass of protoplasm. Within each are two nuclei, known as vegetative and generative nuclei.

In the mass of the pollen protoplasm we observed some corpuscles larger than the granules of protoplasm. These, when treated with iodine, gave the starch reaction.

1. SHAPE AND SIZE OF POLLEN.—The fresh pollen has always a rounded shape; oval, pyriform, elliptical or spherical, and a yellowish green color but, when exposed under natural atmospheric conditions, becomes shrunken and yellow in from ten to twenty minutes.

Although experiments were made with pollen from more than twenty varieties of maize, no uniformity in shape of grains could be determined for any single variety, each variety presenting a mixture of all shapes; pyriform, elliptical, round, etc., with no regularity in the microscopic field.

In some of the varieties we noticed that some one shape would predominate in some degree but a good proportion of all the other shapes were also present in the same variety. In the Roumanian maize called "Common," the predominating shape was oval, in proportion of 60 per cent. The balance was a mixture of round, pyriform and elliptical.

We found variations and mixtures in the shape of pollen not only within the varieties and tassels, but within the stamens belonging to the same tassel. For instance in a tassel of the variety called "Barefoot Pop," we found in a single stamen 60 per cent elliptical and 40 per cent round pollen grains while in another stamen the grains were nearly all oval, and in yet another partly elliptical and oval tapering. Measurements made of more than 1,000 grains of pollen in twenty varieties of maize, show that perfectly spherical pollen is very rare.

In determining the size of pollen, we examined twenty varieties of maize, starting our measurements with pollen from a single stamen in each variety. We found the variation to be relatively large. The greatest variation occurring in the Roumanian variety, "Common," we give the results of our measurements of twenty grains in one stamen in this variety, expressed in "μ."

The dimensions of each grain of pollen is expressed in breadth and length, thus:

116.71 x 139.50
108.50 x 128.34
139.50 x 124.00
108.50 x 124.00
108.50 x 120.90
108.50 x 124.00
108.50 x 124.00
108.50 x 124.00
108.50 x 124.00
108.50 x 120.90
108.50 x 120.90
108.50 x 124.00
108.50 x 124.00
106.17 x 120.90
108.50 x 124.00
108.50 x 124.00
108.50 x 124.00
108.50 x 124.00
106.17 x 120.90
101.37 x 120.90

We also observed the variation in size of the pollen from stamens in different parts of a single tassel, and found that the largest variation occurred also in the Roumanian "Common." The measurements, expressed in "μ" are found in the following table:

TABLE 1. Size of Pollen from Different Stamens in One Tassel.

Stamen No.	No. of grains measured	Upper Central Axis		Middle and Lower Central Axis		Branches	
		Breadth	Length	Breadth	Length	Breadth	Length
1.	10.	106.79	119.19	106.02	121.98	108.50	124.00
2.	10.	104.31	119.19	106.79	116.71	109.89	123.07
3.	10.	107.10	119.19	105.40	120.90	103.69	119.19
4.	10.	108,50	118.26	113.30	128.34	109.12	119.81
Average		106.67	118.95	107.87	121.98	107.80	121.51

From this table which contains the measurements of 120 grains of pollen of twelve stamens from different parts of one single tassel, we found that the pollen from the middle and lower central axis of this tassel was a little larger than that from stamens from the upper central axis and branches, although this order was varied in other tassels, the pollen from the branches being the larger.

In table No. 2, we give the results of the measurements of pollen from different stamens in two tassels of Boone County White Corn, expressed also in "μ."

TABLE 2. Variation in the Size of Pollen in Two Tassels of Boone County White.

Stamen No.	No. of grains Measured	TASSEL 1				TASSEL 2			
		Central Axis		Branches		Central Axis		Branches	
		Breadth	Length	Breadth	Length	Breadth	Length	Breadth	Length
1.	10.	99.20	114.08	94.55	108.50	99.51	108.50	97.65	101.37
2.	10.	99.82	108.81	98.27	111.91	92.22	98.11	96.10	103.54
3.	10.	93.00	107.26	101.68	108.50	94.24	106.17	91.91	106.17
4.	10.	97.34	109.12	97.34	110.05	95.48	102.76	96.56	108.96
5.	10.	99.20	106.95	94.86	111.60	95.79	99.97	104.62	110.20
Average		97.71	109.24	97.34	110.11	95.44	103.10	97.36	106.04

From this table, which contains the measurements of 200 grains of pollen of twenty different stamens from two tassels, we found that there is not a great variation in the size of the pollen from the central axis and branches in one single tassel while there is relatively a larger difference in the size of the pollen in the different tassels.

An interesting study of the size of the pollen of hybrids was made. The pollen, gathered from different stamens of several tassels each of 1857 Bearfoot Sire, 2162 Dam and 2062 Progeny, was measured. As is shown in Table No. 3, the progeny gave pollen larger than that of either parent. This fact, together with other observations concerning the shape of pollen, suggested to the writer that the size and shape of Maize pollen may segregate under Mendelian laws.

TABLE 3. The Effect of Crossing on the Size of Pollen. (Measurements expressed in "μ.")

Stamen No.	No. of grains measured	Central Axis		Branches	
		Breadth	Length	Breadth	Length
1857 Sire (Pop Corn)					
1.	20	95.01	97.65	93.00	98.58
2.	20	92.53	99.51	91.91	95.01
3.	20	92.69	98.11	98.11	104.00
4.	20	91.91	93.00	93.00	102.76
5.	20	87.57	90.83	95.01	97.65
6.	20	93.77	96.10	94.39	102.76
7.	20	94.55	98.89	93.00	100.75
Average		92.57	96.29	94.06	100.21
2162 Dam. (Dent Corn)					
1.	20	101.99	114.39	94.24	105.86
2.	20	107.10	113.61	98.58	115.63
3.	20	89.90	112.53	94.24	105.86
4.	20	85.40	115.01	100.44	109.74
5.	20	106.64	118.73	96.87	105.09
6.	20	104.00	111.75	97.65	102.45
7.	20	93.00	111.60	93.00	102.45
Average		98.29	114.09	96.43	106.72

2062 Progeny. (F1 Generation)

Stamen No.	No. of grains Measured	Central Axis		Branches	
		Breadth	Length	Breadth	Length
1.	20	98.42	107.72	98.42	105.40
2.	20	102.76	111.60	98.89	108.96
3.	20	102.45	109.43	111.60	108.50
4.	20	106.64	114.39	119.81	123.38
5.	20	102.76	110.20	96.25	105.86
6.	20	100.75	107.72	99.35	108.50
7.	20	106.64	109.74	93.00	106.95
Average		102.91	110.11	102.47	109.65

To ascertain the variability of the size of maize pollen an extensive test was made; 1093 grains of pollen, from 21 varieties of maize, were measured, both breadth and length, and the results expressed in "μ," are given as follows:

	Breadth	Length
Mean	96.19±.19	106.04±.21
Standard Deviation	9.33±.13	10.39±.15
Coefficient of Variability	9.70±.14	9.80±.14

2. MOISTURE AND CHEMICAL COMPOSITION: For the determination of moisture content, the pollen was gathered from the field by 8 o'clock in the morning. The tassels were shaken over a large piece of paper and, after all stamens, small insects and any other foreign substances were removed, the pollen was enclosed immediately in weighing bottles and taken to the laboratory for testing. In some samples the moisture was determined at once, while some of the others were stored in a desiccator with water, some in a desiccator with sulphuric acid, and others out of doors, to determine the percentage of moisture lost in two hours, twenty-four hours, and also to ascertain the total moisture of the pollen. The results of these experiments will be seen in Table 4.

TABLE 4. Moisture of the Pollen.

Weight Pollen grams.	Total Dry Matter, grams.	Total Moisture, grams.	Per cent Moisture	Per cent Mois. Lost		Condition of Preservation
				2 hours	24 hours	
1.8625	0.7230	1.1395	61.18	1.23	3.91	In desiccator with water
2.4355	0.9725	1.4630	60.06	1.12	3.71	
1.6660	0.6945	0.9715	58.31	8.91	53.45	In desiccator with Sulphuric acid
1.9685	0.7975	1.1710	59.48	7.39	42.39	
1.4620	0.6362	0.8258	56.48	48.97	52.01	Stored out of doors.
1.6724	0.7234	0.9490	56.74	39.07	51.75	
1.4960	0.7005	0.7955	53.17			Fresh from field
1.5965	0.7435	0.8530	53.42			
3.0560	1.3883	1.6677	54.56			Fresh from field.
1.9300	0.8780	1.0520	54.50			
Moisture Average						56.79 per cent

We found the average moisture of the pollen to be 56.79 per cent, but we believe it to be a little higher as some of the moisture must have been lost by evaporation while the pollen was exposed in cleaning.

The moisture of the pollen varies with the moisture of the air and the maturity of the tassel. Pollen taken from old tassels, on a very hot, dry day, contained only 11 per cent of moisture.

It is interesting to note in Table 4, the results of experiments in the moisture retention of the pollen. One sample stored in a disiccator with water lost 1.23 per cent and another 1.12 per cent in two hours; one stored in a desiccator with sulphuric acid lost 8.91 per cent and the second 7.39 per cent; while the pollen stored out of doors lost, in the same time, 48.97 per cent and 39.07 per cent and after twenty-four hours, 52.01 per cent and 51.75 per cent from a total moisture of 56.48 per cent and 56.74 per cent respectively.

This explains perfectly why the pollen, when manipulated under natural atmospheric conditions, becomes shrunken and soon dies, because it so quickly loses its moisture.

Concerning the chemical composition of the maize pollen, Lidforss (13), states that the pollen contains 4.24 per cent nitrogen and 1.86 per cent phosphorus. According to the same author the pollen of anemophilous plants has a smaller percentage of nitrogen than that of entomophilous plants. Lidforss (13), gives the average of nitrogen in eleven species belonging to anemophilous kinds, including maize, as 4.63 per cent, while for the entomophilous plants the average is 7.49 per cent, with the conclusion that the anemophilous pollen, which must be carried by the wind, is much lighter than that transmitted by insects.

Dr. Smith, of the Illinois University, in an unpublished work, gives the chemical composition of maize pollen as follows:

	Per cent Fresh Substance	Per cent Dry Sub.
Dry Matter	65.16	100.00
Moisture	34.84	0.00
Ash	2.22	3.41
Fat	1.32	2.03
Protein	16.47	25.28
Crude Fiber	5.34	8.20
Carbohydrates	39.81	61.10
Water Extract	27.69	42.50

The writer made an analysis of the nitrogen of pollen in High Protein and Low Protein varieties of maize. These varieties, as is well known, were created at the University of Illinois for the purpose of increasing and decreasing the percentage of protein in maize seed. At the same time, as a comparison, the pollen of Boone County White variety was also analyzed. The results were as follows:

Varieties	Percentage Nitrogen
High Protein	4.82
Low Protein	4.02
Boone County White	4.06

V. GERMINATION OF POLLEN

For the investigation in the germination of the pollen of the maize, the tassels were collected from the field before eight o'clock in the morning, or near the time of the opening of the stamens, and placed in vessels of water on the laboratory desk. By eight o'clock some of the pollen had fallen from the tassels and the rest was gently shaken out on paper ready for use.

The method of germination was that known as the "Hanging Drop Culture." On a glass slide a small glass ring was mounted with paraffine. A drop of the medium used for the germination test was then placed on the cover glass and fresh pollen shaken on this medium by means of a small, soft brush. A little vaseline was rubbed around the edges of the cover glass which was immediately inverted over the ring, a proper label made, and the specimens thus prepared kept under a large glass cover between observations. Records were made after 10, 20 and 30 minutes during the three or four hours of the test. The records given in the following tables are based, not only on duplicated samples, but after often repeated tests.

To avoid any wrong conclusions, the water used was doubled distilled; a glass apparatus being employed for the second distillation.

Maize pollen bursts almost immediately in a drop of water whether it be rain water, distilled or from the tap.

In Table 5., it will be seen that in the different sugar solutions the maize pollen bursts in the first ten minutes although the percentage of bursting decreases somewhat with the concentration of the solution.

TABLE 5. Germination Experiments in Single Solutions.

Solution	CONCENTRATION					
	5%	10%	15%	20%	25%	30%
Sucrose	Bursting	Bursting 20%	Bursting 10%	Bursting 10%	Bursting 4%	Turgid
Lactose	Bursting	Bursting 60%	Bursting 60%	Bursting 60%	Bursting 50%	Bursting 20%
Maltose	Bursting	Bursting 60%	Bursting 60%	Bursting 60%	Bursting 60%	Bursting 20%
Dextrose	Bursting	Bursting 80%	Bursting 80%	Bursting 60%	Bursting 60%	Bursting 20%
Levulose	Bursting	Bursting	Bursting 80%	Bursting 80%	Bursting 70%	Bursting 60%
Arabinose	Bursting	Bursting 90%	Bursting 90%	Bursting 80%	Bursting 60%	Bursting 40%
Glycerine	Bursting 20%	Bursting 20%	Bursting Turgid	Bursting Turgid	Bursting Turgid	Bursting Turgid
Gelatine	Bursting	Pseudo-germ.	Pseudo-germ.	Pseudo-germ.	Pseudo-germ.	Pseudo-germ.
Gum Arabic	Bursting 40%	Pseudo-germ.	Pseudo-germ.	Pseudo-germ.	Pseudo-germ.	Pseudo-germ.

It is a remarkable fact that in a concentration of 30 per cent sucrose, the pollen is turgid with no bursting, while with other kinds of sugar in the same concentration the percentage of bursting is between 20 per cent and 60 per cent. Strong solutions of sucrose and other kinds of sugar, like syrup, were used but the results were the same, the pollen remaining turgid with a small percentage of bursting.

Solutions of gum arabic and gelatin gave very remarkable results which the writer has called "Pseudo-germination." In this medium, during the first ten minutes, the pollen grains were seen to send out long shoots or sprouts of protoplasm which curled and twisted in all directions and with such power as to force the pollen grains backward in the culture. These little sprouts varied in length, in some cases being 25 to 30 times longer than the diameter of the pollen grains. Sometimes they were slender and thread-like; in other cases thicker and stronger. This protoplasm expansion can not be taken for true germination for it lacks most of the necessary characteristics; as for instance the process of expansion is for too rapid to represent actual growth, and again, there is a total absence of the surrounding membrane which is always present encasing the protoplasm, in what is called "tube germination."

It is interesting to note that only the fresh pollen is able to send out these protoplasmic sprouts while the old pollen remains turgid and inactive.

In the microscopic photographs attached, the differences between true germination and what we call pseudo-germination will be very apparent and of deep interest.

Glycerine, corn oil, olive oil, etc., were also tried as media for germination but without results. Decoctions of stigmas, alone or in solution with sugar, as well as those made by grinding the stigmas, gave no results. Daylight, and darkness had no visible effect upon germinations in cultures of sugars, gum arabic, gelatin, etc. Mixtures of sucrose, lactose, maltose, dextrose, etc., in different concentrations produced no results, the percentage of bursting being 20 per cent to 50 per cent.

TABLE 6. Germination Experiments in Mixed Solutions.

Solution	Concentration		Germination
	Simple	Mixture	
Sucrose Lactose	10% 10%	20%	In 20 minutes bursting 50%.
Sucrose Lactose	15% 15%	30%	In 20 minutes bursting 20%.
Sucrose Dextrose	10% 10%	20%	In 20 minutes bursting 20%.
Sucrose Dextrose	15% 15%	30%	In 20 minutes bursting 20%.
Sucrose Lactose Maltose Dextrose	5% 5% 5% 5%	20%	In 20 minutes bursting 50%.
Sucrose Malic acid		20% 0.01%	Bursting 4%; 44% pseudo-germination, protoplasmic expansion 20 times diameter of pollen.
Sucrose Malic acid		5% 0.01%	Bursting.
Sucrose Malic acid		20% 0.05%	Turgid.
Sucrose Citric acid		5% 0.02%	Bursting 50%. Some pseudo-germination.
Sucrose Citric acid		10% 0.02%	Bursting 10%. Pseudo-germination 20%.
Sucrose Citric acid		20% 0.05%	Turgid.
Sucrose Asparagin		15% 0.1%	In 10 minutes bursting 85%.
Sucrose Asparagin		15% 0.5%	In 10 minutes bursting 60%.

Solution	Concentration		Germination
	Simple	Mixture	
Sucrose Lipase		15% 5%	Some bursting, some pseudo-germination.
Sucrose Lecithin		15% 5%	Some bursting, some pseudo-germination.
Sucrose Saltpeter		20% 0.5%	Turgid.
Sucrose Gum arabic	5.0% 5.0%	10%	In 5 minutes bursting 90%.
Sucrose Gum arabic	2.5% 7.5%	10%	In 5 minutes many bursting, some pseudo-germination.
Sucrose Gum arabic	1.0% 9.0%	10%	Bursting 30%; many pseudo-germination.
Sucrose Gum arabic	7.5% 2.5%	10%	In 10 minutes bursting 50%; some pseudo-germination.
Sucrose Gum arabic	9.0% 1.0%	10%	Bursting.
Sucrose Gum arabic	15% 15%	30%	In 10 minutes bursting 80%.
Sucrose Gelatin	5.0% 5.0%	10%	Bursting 30%.
Sucrose Gelatin	2.5% 7.5%	10%	Bursting 20%; Pseudo-germination 10%.
Sucrose Gelatin	1.0% 9.0%	10%	Bursting 20%; some pseudo-germination.
Sucrose Gelatin	7.5% 2.5%	10%	Bursting.
Sucrose Gelatine	9.0% 1.0%	10%	Bursting.

Solution	Concentration		Germination
	Simple	Mixture	
Sucrose Gelatin	15% 15%	30%	Pseudo-germination with very thin and long protoplasmic extension. Bursting 2%.
Lactose Gelatin	15% 15%	30%	In 10 minutes 60% pseudo-germination.
Dextrose Gelatin	15% 15%	30%	In 10 minutes 40% pseudo-germination.
Sucrose Dextrose Lactose Maltose Gelatin	5% 5% 5% 5%	20% 25%	Pseudo-germination 80%, with thin and long protoplasm extension.
Gelatin Malic acid		5% 0.01%	Some pseudo-germination; Bursting 30%.
Gelatin Citric acid		5% 0.01%	Pseudo-germination 50%; Bursting 30%.
Gelatin Sucrose Citric acid		5% 4% 0.01%	In 30 minutes pseudo-germination 94%.
Gelatin Citric acid		5% 0.5%	Bursting 6%. Turgid.
Gelatin Lipase		10% 1%	Bursting 80%; very few pseudo-germination.
Gelatin Saltpeter		10% 0.05%	Turgid.
Gelatin Sucrose Saltpeter		10% 5% 0.05%	Turgid.
Gelatine Asparagin		19% 0.1%	Pseudo-germination 90%.
Gelatin Arabinose		10% 2%	Pseudo-germination 70%.

Solution	Concentration		Germination
	Simple	Mixture	
Gelatin Lecithin		10% 2%	Pseudo-germination 60%, with heavy protoplasm extension.
Gum arabic Glycerine		20% 50%	Pseudo-germination 95 %, Very thin and long protoplasm extension.
Gum arabic Sucrose Citric acid		10% 5% 0.02%	Turgid.
Gum arabic Arabinose		10% 2%	Pseudo-germination 90%.
Gum arabic Arabinose Citric acid		10% 2% 0.01%	Pseudo-germination 40%.
Levulose Lecithin		15% 1%	Bursting; very few pseudo-germination.
Gum arabic Asparagin		10% 0.1%	Pseudo-germination 40%.
Arabinose Lecithin		10% 1%	Bursting 80%; very few pseudo-germination.
Arabinose Malic acid		15% 0.01%	Turgid.
Arabinose Citric acid		15% 0.01%	Turgid.

Malic and citric acid added in the proportion of 0.01 per cent to a sucrose solution of 20 per cent, gave 44 per cent pseudo-germination. Increasing the proportion of acid rendered the pollen turgid, while decreasing the proportion of sugar to 5 per cent with 0.01 per cent of malic acid, resulted in bursting. Lipase and lecithen added to sucrose solutions gave, in many cases, pseudo-germination. Saltpeter added to all sugar solutions produced turgid conditions.

Mixtures in different proportions and concentrations of sucrose and gum arabic or gelatin, gave no valuable results. This was also true when malic or citric acid was used which, when added in more than 0.02 per cent solution in any medium, always produced bursting or turgid conditions. Lecithin added to solutions of levulose and arabinose produced pseudo-germination.

1. REAL GERMINATION: In Table 7 we give the experiments with sucrose and agar which resulted in finding the proper culture to produce real germination.

TABLE 7. Germination Experiments in Solutions of Sucrose and Agar.

Solution	Concentration	Germination
Sucrose Agar	5% 0.15%	Bursting 10%, Turgid.
Sucrose Agar	10% 0.15%	Turgid; Bursting 6%; Germination 5%.
Sucrose Agar	15% 0.15%	Bursting 5%; Germination 5%; Turgid.
Sucrose Agar	20% 0.15%	Bursting 2%; Germination 10%; Turgid.
Sucrose Agar	25% 0.15%	Germination 10%, Turgid.
Sucrose Agar	30% 0.15%	Bursting 2%; Germination 16%. Turgid.
Sucrose Agar	15% 0.020%	Bursting 90%, no germination.
Sucrose Agar	15% 0.030%	Bursting 40%; Germination 6%, Turgid.
Sucrose Agar	15% 0.040%	Bursting 40%; Germination 6%; Turgid.

Solution	Concentration	Germination
Sucrose Agar	15% 0.050%	Bursting 12%; Germination 18%; Turgid.
Sucrose Agar	15% 0.060%	Bursting 6%; Germination 25%; Turgid.
Sucrose Agar	15% 0.080%	Bursting 2%; Germination 15%; Turgid.
Sucrose Agar	15% 0.100%	Bursting 2%; Germination 16%; Turgid.
Sucrose Agar	5% 0.700%	In 10 minutes germinated 4%, burst 8%; after one hour germinated 70%; burst 10%.
Sucrose Agar	10% 0.700%	In 10 minutes germinated 4%. After one hour, 75%; tube long 3-5 times diameter of pollen.
Sucrose Agar	15% 0.700%	Bursting 20%; Germination 20%; Turgid.
Sucrose Agar	20% 0.700%	No germination. Turgid.
Sucrose Agar	25% 0.700%	Turgid.

Agar added in the proportion of 0.015 per cent to different concentrations of sucrose solutions *produced actual germination* of from 5 per cent to 16 per cent. Increasing the percentage of Agar from 0.020 per cent to 0.100 per cent in a 15 per cent sucrose solution increased the percentage of real germination to form 6 per cent to 16 per cent. Agar of 0.700 per cent added to a 5 per cent sucrose solution gave, after one hour, 70 per cent good germination. But Agar of 0.700 per cent in a sucrose solution of 10 per cent gave the best medium for the *real germination of Maize pollen,* which ran as high as 95 per cent in many instances.

The percentage of agar was increased with good results up to 0.75 per cent. Increasing the percentage of agar or sucrose beyond the above limits gave no germinations and only produced bursting or turgidity.

Actual germination of the pollen proceeds slowly. In the medium of agar and sucrose it requires from 30 minutes to one hour for the pollen tube to reach a length of from three to five times the diameter of the pollen grain. In the first ten minutes the intine, through the pore or aperture, always on the side of the pollen grain, pushes outward slightly in the shape of a small bulb. This bulb is covered with a thin membrane and into it migrate the ganules of protoplasm and food reserve from the inside of the grain. Gradually the bulb lengthens into a tube, always encased in its hyaline membrane, and slightly coiling as it proceeds. This is in direct contrast to the procedure in pseudo-germination, where the emergent protoplasm twists and doubles on itself in complex figures. The granules find their way down this tube, always concentrating at the end. The growing period continues for one hour and in this time the tube attains a length of from three to five times the diameter of the pollen grain.

When the pollen tubes have reached their maximum length, so fully have the pollen grains discharged their protoplasmic granules, that a majority of them become partially hyaline and in a few cases, wholly so.

After a period of growth of the pollen tube, the conditions of the moisture, in the drop culture, being changed the pollen tube ceases to grow further.

Within two or three hours after germination of the pollen, the granules of protoplasm which have accumulated in the end of the pollen tube, break through the thin membrane and are thrown out in all directions into the culture medium.

This action produces a general migration of the protoplasmic granules and the pollen grain is quite emptied.

The solutions of sucrose and agar were prepared in small test tubes using double distilled water, and to facilitate the dissolving of the agar, the solution was heated to +100 C., and allowed to cool to from +36 to +40 C., which is the optimum degree for successful germination. Exposed to natural atmospheric conditions, the solution becomes jellied within an hour and has to be re-heated in the test tube with a few drops of the double distilled water added. It is possible thus to make use of the same solution during one day but it does not give very satisfactory results the second day.

To have the best results in germination, the writer wishes to call attention to the necessity of absolute cleanliness of all the vessels and utensils used in the test, especially the slides and cover glasses which must be sterilized against fungi.

In order to establish the efficiency of the solution of sucrose and agar as a germinating medium and also to ascertain the percentage of germination in different varieties of maize, a number of tests were made. Twenty tassels from each of many varieties were collected, the pollen of each variety mixed and subjected to the germination culture.

The results are to be found in Table 8.

TABLE 8. Germination of Pollen of Five Varieties of Maize.

Varieties	Slide No.	No. of pollen grains	Bursting	Germination	Per cent germination	Average % germination	Condition
Leaming	1.	112	6	98	87	80	Tube 4 times diameter of pollen.
	2.	115	2	85	74		
Reid's Yellow Dent	1.	124	10	101	81	77	Same as above.
	2.	118	8	87	74		
Boone County White	1.	120	7	101	84	83	Tube 5 times diameter of pollen.
	2.	100	7	82	82		
Silver Mine	1.	96	2	79	82	88	Same as above.
	2.	142	4	134	94		
Champion White Pearl	1.	151	11	123	81	73	Tube 3 times diameter of pollen.
	2.	124	8	82	65		

From this table we are warranted in our conclusion that the medium specified above is adequate for the germination of pollen of many varieties of maize.

In order to observe the variability in germination of pollen in different tassels in the same varieties, we selected five tassels each of Leaming and Low Yield varieties and after mixing the pollen from each tassel in that variety, prepared two culture slides for each with the results shown in Table 9.

TABLE 9. Variation of Germination of Pollen from Different Tassels.

Tassel No.	Slide No.	No. of Pollen grains	Bursting	Germinated	% of Germination	Average % Germination	Conditions
LEAMING (Variety).							
1.	1.	100	5	82	82	81	Tube 3 times diam. of grain.
1.	2.	140	9	112	80		
2.	1.	122	6	100	81	80	Same as above.
2.	2.	137	5	109	79		
3.	1.	114	10	98	85	82	Tube 5 times diam. of grain.
3.	2.	162	27	130	80		
4.	1.	102	4	91	89	87	Tube 4 times diam. of grain.
4.	2.	119	6	102	85		
5.	1.	109	4	100	91	83	Tube 3 times diam. of grain.
5.	2.	156	20	120	76		
LOW YIELD (Variety).							
1.	1.	143	80	28	19	20	Tube 2 times diam. of grain.
1.	2.	133	69	30	22		
2.	1.	109	44	52	47	41	Tube 3 times diam. of grain
2.	2.	152	55	56	36		
3.	1.	100	5	75	75	81	Tube 2 times diam. of grain.
3.	2.	134	16	118	88		
4.	1.	148	6	140	94	94	Tube 4 times diam. of grain.
4.	2.	134	5	126	94		
5.	1.	120	10	98	81	80	Same as above.
5.	2.	162	22	129	79		

Great care was taken in the selection of the tassels for these tests to have them as uniform as possible in shape, size and maturity. The results obtained in Tables 8 and 9 lead us to the conclusion that the variation in germination of the pollen in different varieties of maize is greater than that from pollen in various tassels belonging to the same variety and that the variation in the germination of the pollen in different tassels of selected and well fixed varieties of maize is very small, while there is a great variation in the germination of pollen from different tassels in varieties of maize which are not selected and fixed. For instance, in Leaming variety, one of the old and well fixed varieties, the germination of the pollen from the various tassels was from 81 per cent to 87 per cent, while in Low Yield, an unselected and unfixed variety, the percentage ranged from 20 per cent to 94 per cent.

VI. THE INFLUENCE OF DRY AND MOIST HEAT ON MAIZE POLLEN

To determine the heat resistance of pollen, many tests were made using both dry and moist oven heat. The results of the experiments are given as follows:

TABLE 10. Dry oven +42 C. Twenty minutes.

Set No.	No. of Pollen Grains	Per cent Germination	Condition
1.	180	0	Burst. 60%
2.	240	0	Burst. 65%
3.	149	0	Burst. 62%
4.	209	0	Burst. 60%
Saturated Atmosphere +42 C. Twenty minutes.			
1.	162	68	
2.	214	69	
3.	156	44	
4.	112	26	
Total	644	207 or 32% germination.	

From the foregoing experiments we determined that fresh pollen, subjected to a dry heat of +42 C., died within 20 minutes, while subjected to the same time and temperature with a saturated atmosphere, obtained by placing a vessel containing water in the oven, a percentage of between 23 per cent and 42 per cent or an average of 32 per cent, of the pollen was still alive.

Pollen was killed in between five and ten seconds in an atmosphere of ether.

VII. THE VITALITY OF POLLEN

The length of time that pollen retains life, after being separated from the tassel, is a very important factor in plant breeding. Much experimenting was done to study the vitality of the pollen under various conditions and the best methods of conserving for the longest possible time its retention of life.

As previously stated, the pollen, under natural atmospheric conditions, loses its moisture in a few hours and in dry heat conditions, is killed in a few minutes. On the other hand, as is shown in the accompanying microscopic photographs, the fresh pollen is moist and preserves its rounded shape while the old pollen, devoid of moisture, is shrunken and dead.

In order to study the length of viability of pollen in the same tassels in any one variety of corn, we selected five tassels of Reid's Yellow Dent, as nearly uniform in size, shape and maturity as possible, and stored them in vessels with water for five days out of doors. The pollen of the tassels was tested each morning with the results given in the following table.

TABLE 11. Vitality of Pollen from Different Tassels of Reid's Yellow Dent.

		First Day		Second Day		Third Day		Fourth Day		Fifth Day	
Tassel No.	Slide No.	% Pollen Shrunken	% Germination	% Pollen Shrunken	% Germination	% Pollen Shrunken	% Germination	% Pollen Shrunken	% Germination	% Pollen Shrunken	% Germination
1.	1.	0	78	27	40	62	Bst-	99	Bst-	99	Tur.
1.	2.	0	84	27	48	62	ing	99	ing	99	Tur.
2.	1.	0	77	14	76	40	22	65	Bst.	100	Tur.
2.	2.	0	85	14	84	40	16	65	ing	100	Tur.
3.	1.	0	82	19	58	49	Bst-	86	Bst-	92	Tur.
3.	2.	0	82	19	66	49	ing	86	ing	92	Tur.
4.	1.	12	60	46	29	71	Bst-	100	Bst-	100	Tur.
4.	2.	12	54	46	12	71	ing	100	ing	100	Tur.
5.	1.	21	46	31	40	55	2	100	Bst.	100	Tur.
5.	2.	21	38	31	42	55	Brs.	100	ing	100	Tur.

From the data given in the above table, we observe that pollen from only two of the five tassels gave a small percentage of germination on the third day, while in all of the other tassels the pollen was dead. No more germination occurred after the third day in any of the tassels.

Another interesting fact was that the percentage of germination decreased every day nearly proportionately with the increasing shrinkage of the pollen in the tassel.

Tests were made with pollen from single stamens in order to ascertain if there was any difference in the germination of pollen in stamens from different parts of the same tassels, but no apparent difference was found.

We also tested the germination of pollen which had remained in some of the stamens after a portion of the pollen had previously been discharged and the percentage of germination of this pollen decreased in proportion as the percentage of skrunken pollen increased in the same stamens.

In many of the stamens, after eight hours, 60 per cent of the pollen was shrunken while the percentage of germination was 22 per cent.

1. STORAGE OF POLLEN. Hundreds of tests were made to determine the vitality of the pollen under natural conditions. Samples of pollen were put into watch glasses in the laboratory desk and out of doors and germination tests made after thirty minutes, one, two, three and four hours, with the general result that, under these conditions, the vitality of the pollen was not retained above four hours. Fresh pollen enclosed in small weighing bottles, under the same conditions, retained its vitality more than 24 hours.

In a preliminary study of the storage of the pollen of Low Yield Variety Maize, the fresh pollen gave 88 per cent germination. Other Conditions gave results as follows:

Stored in	Germination after 4 hours
Sulphuric acid	Bursting, no germination.
Saturated atmosphere	69%. Bursting 28%.
On the desk, uncovered	Bursting, no germination.

The same pollen stored in a saturated atmosphere after 24 hours gave 62 per cent germination and after 32 hours, 30 per cent germination.

In order to know the vitality of pollen under different conditions of moisture, we prepared many desiccators with sulphuric acid pure and diluted in such proportions as to produce 30 per cent, 60 per cent and 90 per cent moisture according to the formula given in the tables of Landolet-Börnstein. This formula was also used by Max Pfundt (19) and is given below:

Sulphuric Acid 15.14% gives 90% Moisture saturation.
Sulphuric Acid 37.69% gives 60% Moisture saturation.
Sulphuric Acid 54.00% gives 30% Moisture saturation.

The pollen which when fresh gave 82 per cent germination, was placed in small watch glasses and stored in these desiccators. Other samples of pollen were stored in the same time on the laboratory desk, and out of doors. The germination was tested in the way and with the results specified in Table 12.

TABLE 12. Storage of Pollen.

	PERCENTAGE OF GERMINATION AFTER					
Stored in:	**2 hours**	**4 hours**	**6 hours**	**20 hours**	**48 hours**	**52 hours**
Laboratory Desk.	**Bursting**	**Bursting**	**Bursting**	**Bursting**	**Bursting**	**Bursting**
Out Doors uncovered.	**42**	**16**	**Bursting**	**Bursting**	**Bursting**	**Bursting**
Desiccator H2SO4	**Bursting**	**Bursting**	**Bursting**	**Bursting**	**Bursting**	**Bursting**
30% Moisture	**48**	**2**	**Bursting**	**Bursting**	**Bursting**	**Bursting**
60% Moisture	**80**	**68**	**55**	**Bursting**	**Bursting**	**Bursting**
90% Moisture	**80**	**80**	**72**	**44**	**22**	**Turgid**
Saturated Atmos.	**77**	**65**	**58**	**49**	**37**	**Turgid**
ANOTHER TRIAL.						
Laboratory Desk	**Bursting**	**Bursting**	**Bursting**	**Bursting**	**Bursting**	**Bursting**
Out doors uncovered	**2**	**Bursting**	**Bursting**	**Bursting**	**Bursting**	**Bursting**
Desiccator H2SO4	**Bursting**	**Bursting**	**Bursting**	**Bursting**	**Bursting**	**Bursting**
30% Moisture	**32**	**Bursting**	**Bursting**	**Bursting**	**Bursting**	**Bursting**
60% Moisture	**74**	**41**	**24**	**Bursting**	**Bursting**	**Bursting**
90% Moisture	**72**	**68**	**45**	**22**	**14**	**Turgid**
Saturated Atmos.	**79**	**71**	**62**	**31**	**30**	**Turgid**

From this table and from the many other tests, we observed that the pollen stored uncovered on the laboratory desk, died in two hours; that out of doors retained its vitality for four hours, while that in the atmosphere with 30 per cent moisture, lived four hours; with 60 per cent moisture, six hours; and with 90 per cent moisture, in a saturated atmosphere, 48 hours. These experiments were repeated many times and with many varieties of pollen with practically the same results.

After 48 hours in the desiccator with 90 per cent moisture and in a saturated atmosphere, the pollen was damaged by fungi.

The reason that the pollen on the laboratory desk died in two hours while pollen from the same tassels, out of doors, retained its vitality for four hours, is found in the difference of conditions of heat and moisture. For instance, during the time of these experiments, the atmospheric temperature in the laboratory registered +24 to +34 C., with a very low percentage of moisture, while out of doors at the same time, the temperature was from +18 to +26 C., with a higher percentage of moisture.

Every practical breeder knows that pollen manipulated in the field under natural conditions, will die in a few hours and he therefore conserves the pollen in paper bags, envelopes or small bottles until ready for use, being sure, however, to use it for pollination in the same day it is gathered.

In our field work when pollen more than six hours old was used, we obtained ears with very many kernels and rows of kernels missing. Sometimes pollen older than six hours and even that which had been gathered for 24 hours and conserved in paper bags, was used and some results obtained. This was possible as in pollination, we used large quantities of pollen for each shoot and the silk not being a laboratory medium, exerted a certain stimulative influence over the weak pollen. But we never obtained full ears or completed rows of kernels under these conditions.

In our hundreds of tests we found that the fresh pollen enclosed in small weighing bottles, kept its vitality for more than 24 hours regardless of external conditions of heat and moisture, because, for that length of time, the pollen was able to retain its moisture in the bottles. After 24 hours, however, it became damaged by fungi.

VIII. THE INFLUENCE OF LOW TEMPERATURE UPON THE POLLEN

The effects of low temperature upon the pollen is very beneficial. We used the ordinary ice box for our experiments, in which we were able to maintain constant temperature of between +10 and +14 degrees C. The pollen was stored in uncovered watch glasses and also in covered weighing bottles. The germination of the pollen in both conditions was tested after four, eight, 24 and 36 hours. We found that although the fresh pollen gave a good percentage of germination, from 80 per cent to 90 per cent, the pollen in the covered weighing bottles not only kept its vitality for 36 hours but the percentage of germination, after 24 hours, had increased to 99 per cent. The uncovered pollen in the watch glasses was dead in six hours. We therefore concluded that, under certain conditions, low temperature may be a stimulent to the germination of the pollen.

We experimented further with pollen which was obtained from suckers late in the summer, between the 20th and 26th of August, and which gave a low percentage of germination. In our previous work we had not been able to detect any appreciable difference in the germination of pollen from the tassels or the suckers which appeared earlier in the season, although the pollen of the late suckers always yielded a smaller percentage of germination.

For these tests the pollen from the late suckers was used and a germination test made. The pollen was then subjected to the effects of low temperature in the ice box with the results given in Table 13. The initial germination was determined very carefully by preparing ten slides from each variety and the average percentage of germination is there shown.

TABLE 13. Influence of Low Temperature Upon the Pollen after 24 hours stored in ice box.

Varieties	Initial Germination	Slide No.	No. of pollen grains	Total germination	Per cent germination
Low Yield	40%	1.	119	111	
		2.	157	152	
		3.	136	130	
		4.	141	134	
			553	527	95%
9359	38%	1.	126	120	
		2.	144	132	
		3.	143	140	
		4.	102	98	
			521	490	90%
9259	34%	1.	165	150	
		2.	142	129	
		3.	150	139	
		4.	102	89	
			559	507	90%
Rice Pop.	58%	1.	100	90	
		2.	110	96	
			210	186	88%

Pollen from the same tassels in the same varieties was stored in the same time and in different conditions of moisture and after 24 hours, germination tests were made. The results are given in Table 14.

TABLE 14. The Stimulative Effect of Low Temperature Upon the Pollen.

Varieties	Initial % germination	PERCENTAGE OF GERMINATION AFTER 24 HOURS				
		Saturated atmosphere	90% moisture	60% Moisture	30% Moisture	Ice Box
Low Yield	40%	Turgid	Turgid	Bst. 50%	Bst. 80%	95%
9359	38%	Turgid	Turgid	Bst. 55%	Bst. 72%	90%
9259	34%	Turgid	Turgid	Bst. 65%	Bst. 80%	90%
Rice Pop	58%	22%	14%	Bst. 50%	Bst. 50%	88%

From the last two tables, we observe that the weak pollen almost lost its vitality after 24 hours under the different conditions of moisture while under the influence of a temperature of +14 C., pollen from the same tassels not only kept its vitality for 24 hours but was stimulated by the low temperature.

We also found that the stimulative effect of the temperature specified, attained its maximum in between 20 and 24 hours after which the percentage of germination decreased and so we registered after 48 hours, a 29 per cent germination for Rice Pop variety and 12 per cent for the other varieties. Pollen stored in the ice box at +14 C., temperature, gave no more germination after 48 hours.

IX. PRACTICAL STORAGE

Parallel with this work the writer tried some of the experiments in practical storage of pollen, also used by other investigators.

In a test tube was placed a small amount of fresh pollen. The tube was hermetically sealed with a cork to which was affixed a sponge moistened with water and separated from the pollen by a piece of blotting paper. Tests were repeatedly made and the vitality of the pollen remained good after 24 hours, and a good percentage of germination was found after 36 hours, although after that time the pollen was damaged by fungi.

Small branches were cut from tassels early on the morning in which they were about to bloom and conserved in a desiccator with water in the laboratory. It was found that the discharge of the pollen was thus retarded for 24 hours and we had from the stamens the next morning, fresh pollen with a good percentage of germination. In order to further retard the blooming of the pollen, some of the branches were first put in a desiccator with sulphuric acid and left for different lengths of time. They were then placed in desiccators with water but the results were not satisfactory as the dry atmosphere wilted the flowers and but a very few of them continued blooming.

Another test to retard the maturity of the tassels was made by taking fresh tassels, each carefully wrapped in its own leaves and all bound together in a tight package with paper and string, and placed out of doors in the shade, The vitality of the pollen remained good after 24 hours, although greatly reduced after 48 hours. Some of the tassels were sprinkled with water but did not give good results as the extra moisture favored fungi.

X. CONCLUSIONS

In view of the foregoing experiments, we have drawn the following conclusions:

1. That the Maize pollen, when fresh, is of regular shape and has a smooth surface while old pollen is shrunken and rough, probably due to the rapid escape of water as shown by quantitative determination of moisture.

2. That the chemical composition of the pollen seems to be influenced by selection for protein in the kernels, the analysis showing a correlation.

3. That from the material investigated there is evidence of an increase in size of pollen in an F_1 generation cross due probably to the influence of heterozygosis.

4. That in certain media, Maize pollen throws out a protoplasmic expansion; this behavior we have designated as "Pseudo-Germination," and is not to be taken as representing the true germination process.

5. That there is considerable difference in the germination of pollen of different varieties of Maize.

6. That there is a great variation in the germination of pollen from different tassels of an unselected or unfixed variety of Maize. This suggests to the writer the possibility of selecting, in hand crossing work, pollen from tassels which gives the largest percentage of germination.

7. That dry heat is injurious to the vitality of the pollen, while moist heat can be satisfactorily resisted.

8. That the percentage of germination decreases proportionally to the increase of the shrunken pollen.

9. That pollen exposed in the laboratory died in two hours; uncovered out of doors it lived four hours; in 60 per cent moisture it lived for six hours and in 90 per cent moisture, or in a saturated atmosphere, for 48 hours. The same pollen practically hermetically sealed in small tubes or bottles kept its vitality for 24 hours regardless of external conditions.

10. That pollen from early suckers is just as viable as pollen from the parent plants, but that from late suckers gives a low percentage of germination.

11. That low temperature (from +8 to +14 C.,) has a stimulative effect upon the vitality of the pollen, including that from late suckers.

12. That as pollen under natural atmospheric conditions will die in a few hours, the practical breeder should use the pollen, if possible, on the day in which it is gathered; but if the stigmas are not receptive on that day, the pollen may be stored in small tubes or bottles for 24 hours, and still be capable of fecundation, or if the pollen is weak, its vitality can be stimulated by subjection to the effects of low temperature.

XI. APPENDIX

This thesis must be considered merely as the initial chapter in the study of the fecundation of Maize, and the writer hopes to be able to contribute additions to the work already done as a result of future investigations.

The writer also wishes to express his gratitude for the opportunities for study which have been afforded him by the University of Illinois and his appreciation of the splendid privileges of laboratory and research which he has enjoyed, together with the unfailing courtesies and valuable assistance given him by every one connected with the University.

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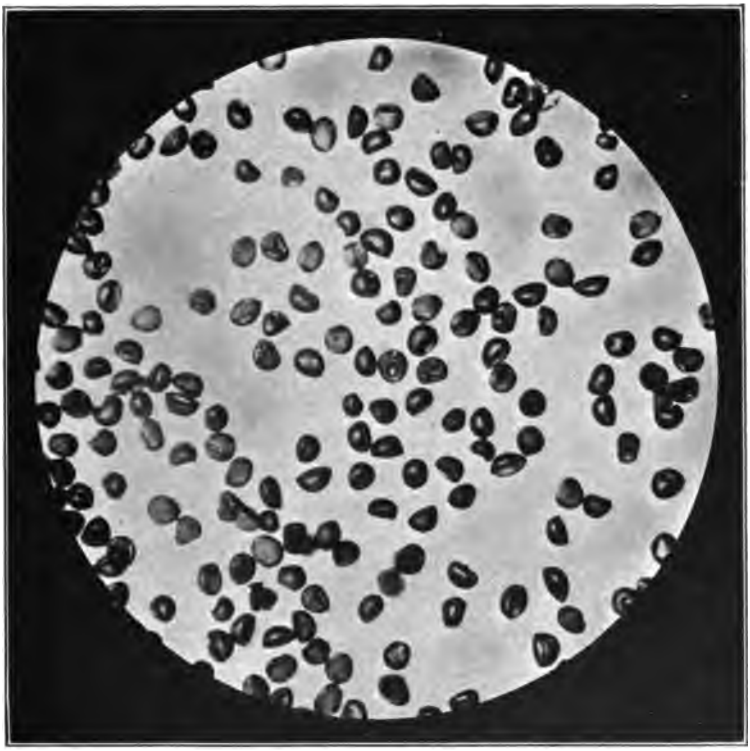
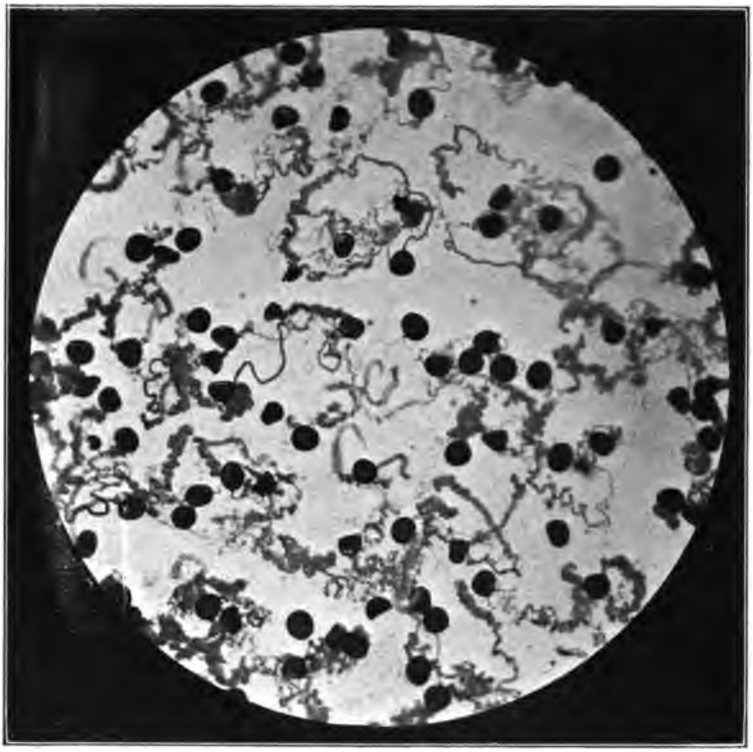
XIII. PLATES

PLATE I



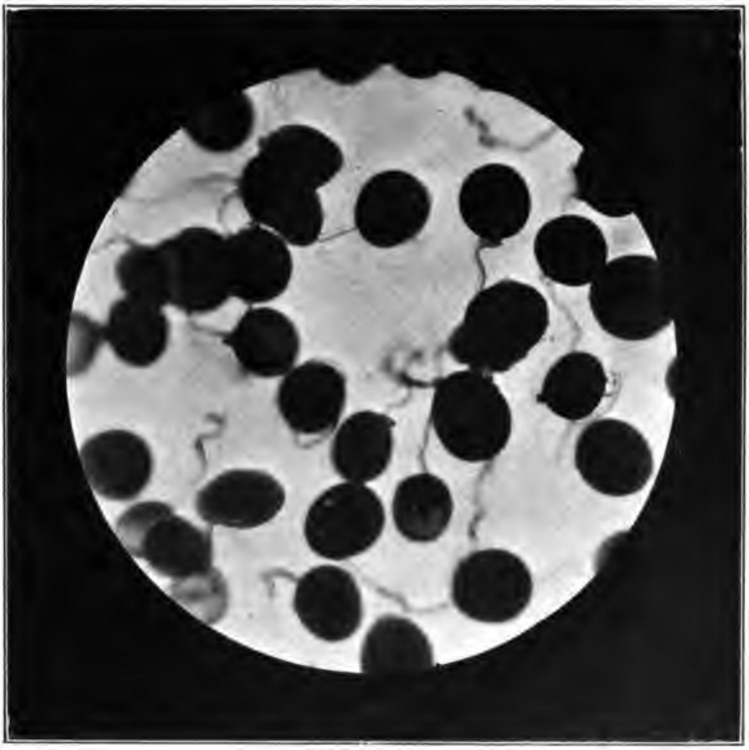
Pollen of Maize as it looks after half an hour's exposure under natural atmospheric conditions. In this shrunken condition the percentage of germination is greatly reduced and the pollen soon dies. Enlargement 50 times.

PLATE II



Pseudo-germination of Maize Pollen, obtained in ten minutes in solutions of Gum Arabic. Similar results may be obtained with gelatin solutions. Enlargement 50 times.

PLATE III



Germination of Pollen of Maize as it looks after three hours. Percentage of germination 95 per cent. Note the effect of the protoplasm granules through the pollen tubes and spreading out into the culture medium. Enlargement 100 times.